Elastin-like Polypeptides (ELPs) are genetically engineered biopolymers that are derived from the endogenous protein Tropoelastin. ELPs are structurally disordered and soluble at low temperatures but transition to a β-spiral and aggregate at a Transition Temperature (T_T). This aggregation is being explored as a novel drug delivery vector by thermally targeting systemically delivered ELP-drug conjugates. We are investigating the biophysical properties of ELP [$V_5G_3A_2$-150] as a means of understanding and predicting the behavior *in vivo*. We have investigated the hydrodynamic, structural and thermodynamic properties of ELP[$V_5G_3A_2$-150] through the use of CD, turbidity, DLS, DSC and SV. DLS and SV analyses suggest that ELP[$V_5G_3A_2$-150] experiences small amounts of weak association below the T_T that increase with temperature. CD analyses further indicate that below the T_T ELP[$V_5G_3A_2$-150] consists of both disordered ($\approx$75%) and β-conformation ($\approx$25%) and as the temperature and concentration is increased the % β-conformation increases. The temperature & concentration dependence of β-conformation suggests that the weak association can be attributed to heterogeneous β-sheets. SV revealed that above the T_T ELP[$V_5G_3A_2$-150] exhibits a temperature dependent critical concentration (C_C). This C_C is consistent with the T_T and suggests that the T_T may be described as a solubility constant.
Assembly in serum raises the T_T by $\approx$ 2.5°C. This is opposite to the expected effect of macromolecular crowding and suggests that certain serum proteins may be associating with ELP[$V_5G_3A_2$-150]. Investigation of this effect through SV was greatly complicated by the presence of the Johnston-Ogston (J-O) effect. Further investigation suggested additional complexity in systems exhibiting the J-O effect than previously reported. Two of the additional complexities already determined are cross-term hydrodynamic non-ideality and high-concentration convection. Additional research into the effects of attaching CPPs to ELP[$V_5G_3A_2$-150] will be presented. Work supported by NSF ARRA 0959211 grant.

2839-Plat
Elucidating the Dimer Interface of SecA
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SecA is an ATPase that mediates preprotein translocation through the SecYEG channel. SecA is a potential target for antibacterial therapeutics because it is crucial for protein transport and cell viability, it is highly conserved among species of bacteria, and it has no close human homologs. As a central component in the general secretion pathway of bacteria, SecA interacts with various ligands, including other SecA molecules. SecA exists in a monomer-dimer equilibrium at micromolar concentrations that is highly sensitive to salt concentration and temperature. Although the structure of the SecA protomer is well-conserved among bacterial homologs, multiple dimer interfaces have been identified. To define the physiological dimer interface of SecA, we have performed site-directed mutagenesis based on the alternative dimer interfaces reported in the crystal structures. Residues for mutagenesis were chosen by computational alanine scanning using the program Robetta. The selected mutations were predicted to destabilize the interface by at least 1 kcal/mol. By using sedimentation velocity, we determined the effect of alanine substitution on dimerization energetics. We have identified four residues that substantially affect dimerization.

2840-Plat
Early Events of the Heat Shock Response
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The 70-kilodalton heat shock protein (hsp-70) is central to the cellular stress response: almost all organisms express an hsp-70 analog. Hsp-70 is thought to protect the cell against stress-initiated protein denaturation and aggregation by binding hydrophobic residues exposed upon protein unfolding. Despite decades of research using biochemical and biophysical techniques, the exact mechanism with which hsp-70 interacts with its co-chaperones and selects its target proteins is still unknown. We use FRET fluorescence microscopy to watch fluorescently labeled hsp-70 bind unfolding protein targets with spatial and temporal resolution both *in vitro* and in live cells. Proteins are unfolded *via* steady state heating and infrared temperature jump with a time resolution of 50 ms. We find that hsp-70 binds a destabilized mutant of phosphoglycerate kinase (PGK) at ~40°C *in vitro* and in cells, which is very close to the protein melting temperature. We also find that when temperatures are increased up to 50°C, hsp-70 does not bind a stable PGK mutant, which melts at 55°C. Other tested protein substrates are also bound by hsp-70 in a melting temperature dependent interaction in cells. These results and future experiments will help us shed light on the molecular mechanism used by hsp-70 to protect the cell against protein denaturation and aggregation.

2841-Plat
Effects of Molecular Crowding on the Repeat Domain of a Functional Amyloid, Pmel17
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The repeat domain (RPT) of Pmel17 is a functional amyloid that contributes to the fibrillar matrix observed in early stage melanosomes. These internal fibrils serve as a scaffold to polymerize and store the pigment, melanin. The aggregation and fibrillation propensity of RPT occurs only at the mildly acidic pH (4.5-5.5), typical of melanosomes. However, these in vitro studies do not represent the crowded and viscous conditions in vivo. To better understand how intraorganellar environment might affect RPT aggregation, we used various types of polymers, such as neutral polyethylene glycols and polysaccharides (e.g. Ficolls) to simulate these conditions. We find that molecular crowding agents dramatically accelerate amyloid formation as well as change fibril morphology. Interestingly, the critical pH-regime for RPT was altered, with aggregation occurring at pH > 6. These results demonstrate that RPT is highly amyloidogenic when subjected to in vivo-like conditions.

2842-Plat
LC3 Constitutively Associates with a High Molecular Weight Complex in Both the Cytoplasm and Nucleus
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LC3 is a key component of the autophagy pathway, where it functions in autophagosome formation and cargo recruitment. Recent proteomics analysis indicates LC3 and other members of the ATG8 family interact with 67 different proteins, many of which appear to bind to common sites on LC3. However, it is currently unknown how many proteins are bound to LC3 at a given time, or if cytoplasmic and nuclear forms of LC3 interact with similar or different proteins. Here, we report several independent lines of evidence that suggest LC3 is constitutively associated with a high molecular weight complex in both the cytoplasm and nucleus. First, confocal FRAP measurements indicate that Venus-LC3 diffuses significantly more slowly than Venus alone in both the cytoplasm and nucleus of living cells. Second, fluorescence correlation spectroscopy measurements of freshly prepared cell homogenates report the correlation time of Venus-LC3 is longer than that of Venus alone. Third, both cytoplasmic and nuclear forms of endogenous LC3 and Venus-LC3 migrate as part of a high molecular weight complex in blue native gel electrophoresis. To test whether multiple copies of LC3 are contained within the same complex, we performed FRET analysis between Venus- and Cerulean-tagged LC3, homoFRET analysis of Venus-LC3, and brightness analysis of Venus-LC3. No FRET was detected between LC3 molecules in the cytoplasm or in cell homogenates, and the brightness of LC3 was consistent with approximately 1 Venus-LC3 per complex. However, a small level of FRET was measured between LC3 molecules in the nucleus, suggesting multiple copies of the protein may be present within the same complex. Experiments are currently underway to determine how the formation of these complexes is regulated as well as to identify other complex components.

2843-Plat
Unraveling the Link between Molecular Conformation and Morphology and Mechanics of Amyloid Fibrils
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Nearly all proteins and peptides have the ability to self-assemble into amyloid fibrils when they are denatured. These highly ordered nanofibrils exhibit superior mechanical properties, and are therefore attractive candidates for applications in materials science and food industry. The flipside of the remarkable stability is their accumulation in tissues in the context of conformational diseases.
It is thought that the high stability and rigidity of amyloid fibrils is caused by β-sheets, which are stabilized with hydrogen bridges. However, spectroscopic measurements show that amyloids contain not only β-sheets, but also have a pronounced α-helical and random coil content, and morphological measurements show that amyloids are highly polymorphic. The link between molecular conformation and the mesoscopic fibril structure and mechanical rigidity is still not understood. Our strategy to elucidate this link is to measure both the mechanical properties and the molecular structure of amyloid fibrils prepared from the model protein β-lactoglobulin (β-lg).